





Product Sheet

ROCK Inhibitor Y27632 (ATCC® ACS-3030™)

Please read this FIRST



Storage Temp.
-20°C to -80°C



Biosafety Level
*

Description

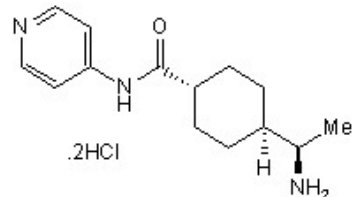
Product Description:

Volume: 10 mg

Storage: -20°C

ROCK Inhibitor Y-27632 is a cell permeable potent and selective inhibitor of the Rho-associated protein kinase (ROCK). Treatment with ROCK Inhibitor Y-27632 increases the survival of many types of stem cells *in vitro*. This product has been qualified for use with 3D culture of organoids, hESCs/hiPSCs, and conditionally reprogrammed cells (CRC).

Structure of ROCK Inhibitor Y27632



Chemical name: *trans*-4-[(1*R*)-1-Aminoethyl]-*N*-4-pyridinylcyclohexanecarboxamide dihydrochloride

Volume: 10 mg

Directions for Use

Preparation and storage

Prepare a 10 mM stock solution

1. Aseptically add 3 mL of sterile water (ATCC 60-2450) or D-PBS (ATCC 30-2200) to the 10 mg vial of ROCK Inhibitor Y27632. Mix thoroughly by pipetting.
2. Aliquot the stock solution in working volumes based on routine use.
3. Store aliquots at 20°C to 80°C and avoid repeated freezing and thawing. Once thawed, aliquots may be kept at 2°C to 8°C for two weeks.

A. Organoid culture

- Use at a final concentration of 10 µM (1 µL of a 10 mM stock solution per mL of complete culture media).
- Supplement the media for the first 2-3 days post-thaw and during the first 2-3 days post-passaging.
- Add ROCK Inhibitor Y-27632 directly to complete culture media as needed. Do not store culture media once supplemented.

B. Conditional reprogramming of cells (CRC) culture

- Utilized as a component in F-media. Use with or without feeder cells.
- Use at a final concentration of 10 µM (1 µL of a 10 mM stock solution per mL of complete culture media)

C. Human induced pluripotent stem cells (iPSC) culture

Refer to the [ATCC Stem Cell Culture Guide](#) for more information.

Cell culture medium: Pluripotent Stem Cell SFM XF/FF (ATCC ACS-3002) is recommended for feeder free culture and ROCK Inhibitor Y-27632 is required for initial culture of iPSCs.

Preparation of Medium Supplemented with ROCK Inhibitor Y-27632

1. Thaw 10 mM stock solution of ROCK Inhibitor Y-27632 on ice.
2. Dilute 10 mM ROCK Inhibitor Y-27632 1:1000 in cell culture medium to obtain a final concentration of 10 µM. For example, if you are preparing 500 mL of media add 0.5 ml 10 mM ROCK inhibitor
3. Medium supplemented with ROCK Inhibitor Y-27632 must be used immediately.

Handling Procedure for Frozen Cells and Initiation of Cultures

1. 30 Minutes Prior to Handling Cells Prewarm the appropriate stem cell culture medium at 37°C for at least 30 minutes before adding to cells.
2. Remove cryovial of frozen cells from liquid nitrogen storage.
3. Thaw the cells by gentle swirling in a 37°C water bath. To reduce the possibility of contamination, keep the Oring and cap out of the water. Thawing should be rapid (approximately 1 to 2 minutes). Remove the cryovial from the water bath when only a few ice crystals are remaining.
4. Sterilize the cryovial by rinsing with 70% ethanol.
5. Using a 1 mL or 5 mL pipette, gently transfer the cell suspension to a 15 mL conical tube.
6. Slowly add 4 mL stem cell culture medium including 10 µM of ROCK Inhibitor Y-27632 (ATCC ACS-3030) dropwise, to the conical tube. Use an additional 1 mL of media to rinse the cryovial and transfer the liquid to the 15 mL conical tube. Shake the conical tube gently to mix the cells while adding media.
7. Gently pipette the cells up and down 1-2 times to mix thoroughly. Avoid breaking apart the aggregates into a singlecell suspension.
8. Centrifuge the cells at 200 x g for 5 minutes.

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
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The graphic is a black rectangle with white text and icons. At the top left is a white thermometer icon. To its right, the text reads 'Storage Temp. -20°C to -80°C'. Below this is a horizontal dashed line. At the bottom left is a white biohazard symbol. To its right, the text reads 'Biosafety Level *'.

9. Aspirate the supernatant and discard. Gently tap on the bottom of the tube to loosen the cell pellet.
10. Add 1 mL of stem cell culture medium that has been supplemented with ROCK Inhibitor Y27632 (ATCC ACS-3030) to a final concentration of 10 μ M. Gently resuspend the pellet by pipetting up and down 1-2 times with a 1 mL tip, maintaining the cell aggregates.
11. Plate the cells as desired under feederfree culture condition
12. ROCK Inhibitor Y-27632 is not necessary in subsequent cell culture medium changes and is not required for passaging cells.

Note: The use of ROCK inhibitor may cause a transient spindle-like morphology effect on iPSCs. However, the colony morphology will recover after subsequent media change without ROCK inhibitor

References

1. Li X. et al, ROCK inhibitor improves survival of cryopreserved serum/feeder-free single human embryonic stem cells. Hum Reprod 24(3): 580-589, 2009. PubMed: 19056770
2. Liu, Xuefeng, et al. ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells. Am J Pathol 180(2): 599-607, 2012.

Quality Control Specifications

Purity is > 98% as confirmed by HPLC analysis. Structure and mass are confirmed by NMR and mass spectrometry. A Certificate of Analysis is available upon request.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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